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TITLE: Vasopressin Regulation and Renal Fluid and Electrolyte Handling in Rat Model of Acute and Chronic Alcohol Exposure

PRINCIPAL INVESTIGATOR: Catherine F. T. Uyehara, Ph.D.

CONTRACTING ORGANIZATION: Tripler Army Medical Center
Tripler AMC Hawaii 96859-5000

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6. AUTHOR(S) :
Catherine F. T. Uyehara, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Tripler Army Medical Center
Tripler AMC Hawaii 96859-5000

e-mail: catherine.uyehara@amedd.army.mil

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13. ABSTRACT *(Maximum 200 words)*
We have previously shown that after effects of short term alcohol exposure cause rats to have an increased water diuresis in response to a water load whereas after long term alcohol exposure, rats exhibit an impaired ability to excrete a water load. Our latest data indicate that in a 4-week withdrawal phase from chronic alcohol exposure, water excretion impairment begins to return towards control levels. The up- and down-regulation of renal vasopressin V2 receptors appear to be behind differentially altered renal function in different phases of alcohol exposure. Prolonged diuresis during the acute phase of alcohol exposure is associated with a decrease in V2 receptor mRNA and a down-regulation of V2 receptors. During chronic alcohol exposure, renal V2 receptor mRNA is up-regulated, indicating that increased synthesis of V2 receptors causes increased renal efficacy of vasopressin despite similar circulating vasopressin levels between alcohol-exposed and control rats. During the withdrawal phase, V2 receptor mRNA returns toward normal levels, demonstrating that chronic alcohol-induced impairment of renal water excretion is reversible. Results suggest that alcohol-induced changes in renal responsiveness to vasopressin causes the pattern of diuresis, impaired water excretion, and recovery in the different phases of alcohol exposure.

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INTRODUCTION:

Alcohol use impairs renal fluid handling and the ability to maintain adequate hydration. Of importance from a military readiness aspect, is that alcohol exposure causes physiological changes in fluid and electrolyte balance that will affect soldier performance. The soldier who uses alcohol is even more susceptible to dehydration especially when water is scarce, and from a pharmacological perspective, would be more susceptible to exposure to chemical warfare agents that would reach toxic levels in the dehydrated alcohol user faster than an individual with adequate hydration. A better understanding of renal fluid and electrolyte handling in response to alcohol is needed to design better treatments in dealing with fluid and electrolyte imbalances seen in alcoholics. Fluid and electrolyte balance appears to be affected differently at different stages of alcohol use. In this study, the role of vasopressin, an important hormone in body fluid regulation, in the physiological response to alcohol is being examined. In rat models of acute and chronic alcohol exposure, we are taking a systematic approach at elucidating the relationship between vasopressin synthesis in the brain, receptor regulation in the kidneys, and water and salt handling during different phases of alcohol exposure. In this second year of this study, we have investigated the renal mechanisms behind the increased diuresis after acute alcohol exposure and impaired water excretion seen after chronic alcohol exposure. The results of this research will lead to better strategies for management of fluid and electrolyte imbalance associated with alcohol use and will benefit military operational readiness by helping to provide medical countermeasures for soldiers who use alcohol.

PROGRESS IN YEAR 2:

Good progress continues to be made during the second year of this project. We have continued whole animal renal function experiments in our now well-characterized animal models of different phases of alcohol exposure and have added a model for alcohol withdrawal. In addition, we have developed new real-time polymerase chain reaction (qPCR) assays for quantitation of mRNA for vasopressin and vasopressin receptor syntheses. During this second year, we have focused on studying the mechanisms involved in the differentially altered renal function and fluid balance effects in the different phases of alcohol exposure. The major specific tasks of 1) evaluating fluid and electrolyte regulating ability in models of acute and chronic alcohol exposure and alcohol withdrawal, and 2) searching for mechanisms of altered fluid handling via *in vitro* assessment of vasopressin receptor levels in the brain and kidneys are underway and beginning to yield exciting data. Research accomplishments associated with each task outlined in the Statement of Work are as follows:

1. *Fluid and electrolyte regulating ability experiments*

Animal models: We have established dependable animal models of acute and chronic alcohol exposure and now alcohol withdrawal. Chronic alcohol and withdrawal models are on liquid diets that allow fixed fluid and electrolyte intake so that changes in fluid balance are due to fluid and electrolyte output alterations. All models developed can be studied repeatedly in several experiments, resulting in a considerable reduction of the total numbers of animals used in this study (about 25% of the original predicted numbers). We continue to take advantage of the ability to run several experiments in the same animal. This allows for control of variability between individual animals, and enables the generation of data with greater precision and detection of finer differences in physiologic regulation of fluid balance between groups. This repeated measures design also provides for closer comparison of several arms of the experiments and simultaneous assessment of multiple aspects of fluid regulation.

Examination of the ability of the kidneys to excrete a water load:

Acute and chronic alcohol effects: Experiments testing the ability to excrete a water load after either acute or chronic alcohol exposure have been completed. We have confirmed our previous report of acute alcohol exposure increasing water diuresis over 18 hours after the last alcohol intake, even after blood alcohol levels are undetectable. Likewise, the impaired ability to excrete a water load of rats with chronic alcohol exposure has been verified. The mechanisms behind altered renal water handling ability could be a difference in vasopressin circulating levels as a result of changes in the regulation of vasopressin synthesis, release, or metabolism, and/or a difference in renal responsiveness to vasopressin. Thus in this second year, as described under Specific Task #2, we have focused our attention on the *in vitro* assessments of brain synthesis and kidney receptors to elucidate the mechanisms involved in altered water handling.

Withdrawal from chronic alcohol effects: Rats chronically exposed to alcohol for 8 weeks via liquid diet were studied 4 weeks after removal of alcohol from their diet. Results indicate that there is a reversal of the impaired water load excretion ability seen during chronic alcohol exposure. As can be seen in figure 1A, the time course profiles of water load excretion in rats in the withdrawal group were similar to that in the control group with peak water load excretion occurring within 30 minutes after water load. In contrast to the water load excretion in

the acute and chronic alcohol exposure groups, however, water load excretion ability returns toward control levels and is not statistically different between the withdrawal and control groups (fig. 1B). This agrees with studies examining other aspects of organ function that suggest that alcohol induced organ impairment is reversible (Ishigami et al, 1997; Silva et al, 2002.)

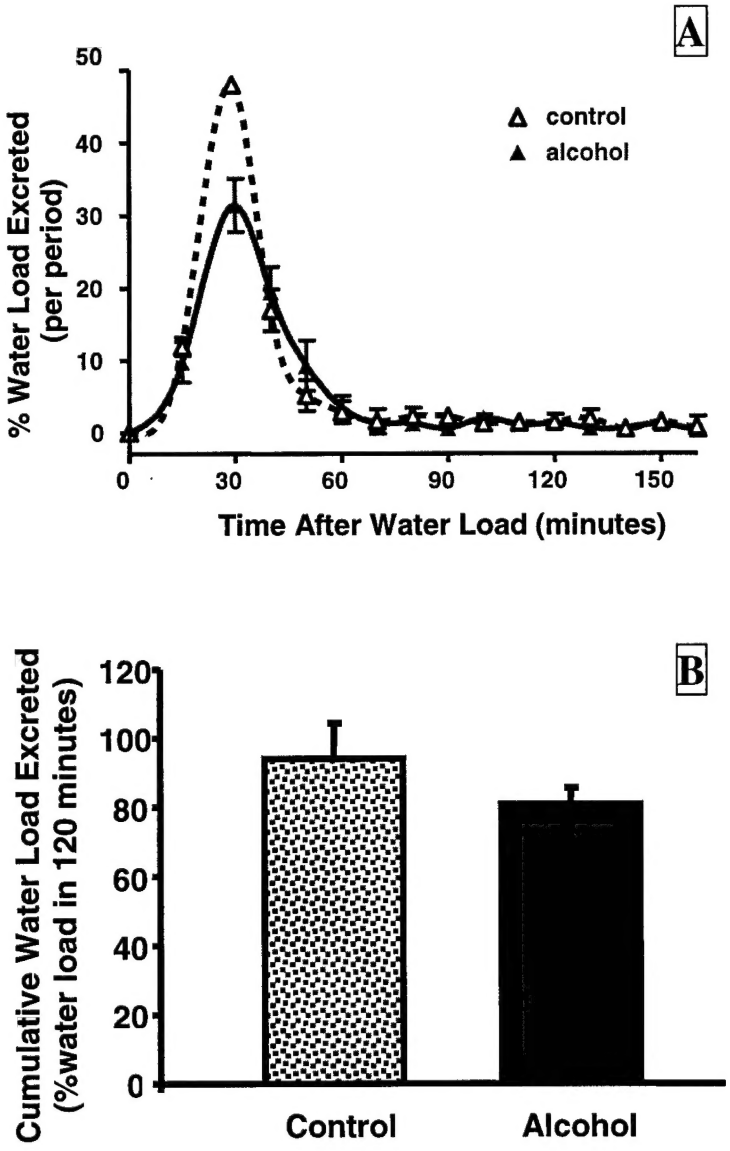


fig. 1 Withdrawal from alcohol effects on water load excretion ability.

As observed in rats with acute or chronic alcohol exposure, time course of water load excretion profiles in rats 4 weeks after withdrawal from chronic alcohol (n=5) were similar to the excretion profiles in the control group (n=5). In contrast to the enhanced water load excretion seen in the acute alcohol exposure group and the substantially impaired water excretion seen in the chronic alcohol exposure group, there was no significant difference in the total water load excreted after 120 minutes between control and alcohol withdrawal groups. Values represent means $\pm$ s.e.m.

V2 antagonist dose response curve generation to examine the renal response to endogenous vasopressin:

We postulated that alcohol may alter renal handling of fluid in acute and chronic alcohol use by affecting regulation of renal V2 receptors involved with tubular water reabsorption. To examine the whole animal effects of putative V2 receptor up or down regulation, we have completed V2 receptor antagonist dose response experiments in acute alcohol exposure, chronic alcohol exposure, and alcohol withdrawal models.

Acute alcohol effects: V2 antagonist induced similar changes in urine flow, urine osmolality, free water clearance, and osmotic clearance in control rats and rats with short term alcohol exposure. Hence, these results indicated that in acute alcohol exposure the kidneys may have not yet adapted with a long-lasting change in renal sensitivity to acute changes in endogenous vasopressin levels, or that receptor down regulation is simply inherently difficult to detect with antagonist administration.

Chronic alcohol effects: In accordance with their impaired ability to excrete a water load, rats chronically exposed to alcohol showed a blunted diuresis and a rightward shift of the dose-response curve to V2 antagonist inhibition of endogenous vasopressin. The suppression of V2 antagonist efficacy in increasing urine flow was due to attenuation of free water clearance in the chronic alcohol group.

This decrease in V2 antagonist efficacy occurred despite no apparent differences in plasma vasopressin levels in these rats. Such results are consistent with the hypothesis that impaired ability to excrete a water load and a SIADH-like phenomenon of water retention in chronic alcohol users are due to altered renal responsiveness to endogenous vasopressin. It is possible that an up regulation of vasopressin receptors in the face of long-term alcohol exposure, similar to that seen with long-term exposure to vasopressin antagonists (Caltabiano and Kinter, 1991) may occur due to lasting changes in steady state circulating levels of vasopressin, or possibly due to direct actions of alcohol itself. Thus, to compensate for the initial acute alcohol-induced diuresis effect, an up regulation of vasopressin receptors in the kidney occurs. When eventual compensation by the brain to increase vasopressin synthesis to restore normal vasopressin circulating levels occurs in the steady state chronic alcohol exposure phase, the kidney is hypersensitive to vasopressin due to the up-regulation of renal receptors.

Withdrawal from alcohol effects: Similar to V2 antagonist dose response curves generated in rats during chronic alcohol exposure, there is a slight decreased maximal urine flow (fig. 2A) achieved with V2 antagonism due to a blunting of free water clearance (fig. 2C) and no change in osmotic clearance (fig. 2D). This attenuation is seen only at the maximal efficacy of the dose response curve and not an overall shift of the curve to the right as previously seen in the chronic alcohol exposure group, indicating similar renal V2 sensitivity and a return towards control responses. The decrease in efficacy may instead be due to changes in the renal environment for water reabsorption. Interestingly, withdrawal from alcohol appears to result in a slight increase in baseline urine osmolality (fig. 2B) but not in maximal urine dilution capacity. The increased basal urine osmolalities seen during alcohol withdrawal suggest that while the

kidney is starting to return towards normal, the kidneys are still holding on to more water than controls as adjustments in renal responses to vasopressin are being made.

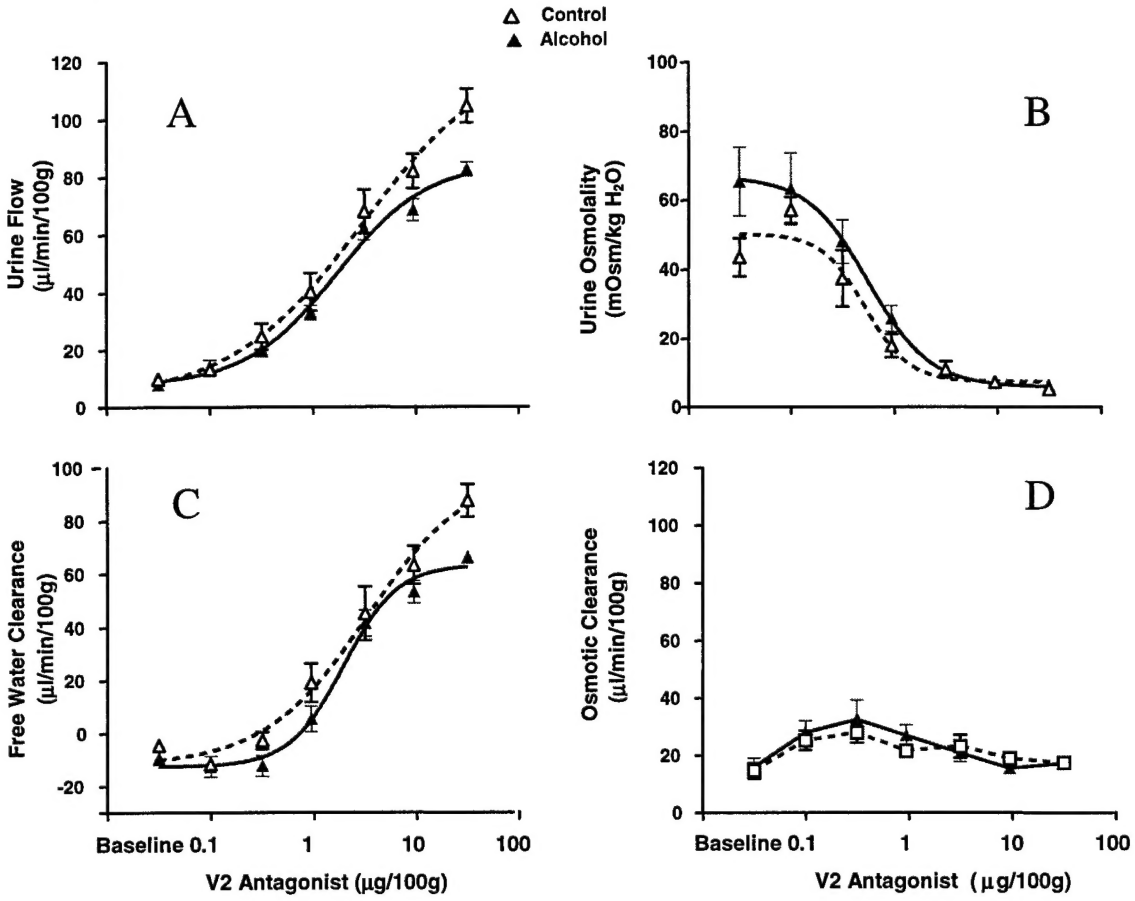


fig. 2. Withdrawal from alcohol exposure effects on response to V2 antagonist. In contrast to chronic alcohol exposure causing a shift to the right of the V2 antagonist dose response curves for urine flow and free water clearance, during withdrawal from alcohol, the response to a V2 antagonist returns towards control responses, although maximum efficacy in withdrawal group (n=5) is less than that of control group (n=5).

V2 agonist dose response curve generation to assess maximum urine concentrating ability with maximal stimulation of vasopressin V2 receptors:

Because altered water handling in alcohol exposed rats may be due to an alteration of the renal medullary interstitium tonicity in these animals, the urine concentrating abilities in the face of maximal vasopressin V2 receptor stimulation in these rats were examined. dDAVP dose-response experiments so far show that there is no difference in maximal urine concentrating ability between the rats chronically exposed to alcohol (n=6) and control rats (n=5). Thus, these results suggested that the rats do not have an altered renal tonicity for fluid reabsorption, and it was likely that the altered fluid handling observed after chronic alcohol exposure was primarily due to differences in V2 receptor density.

However, the maximum urine concentration ability in response to maximal V2 stimulation with dDAVP is lower in rats experiencing alcohol withdrawal than in control rats

(fig. 3). This suggests that during this phase of withdrawal, renal medullary tonicity is altered. Further, preliminary data indicate that this change in renal medullary interstitium tonicity is due to altered urea excretion.

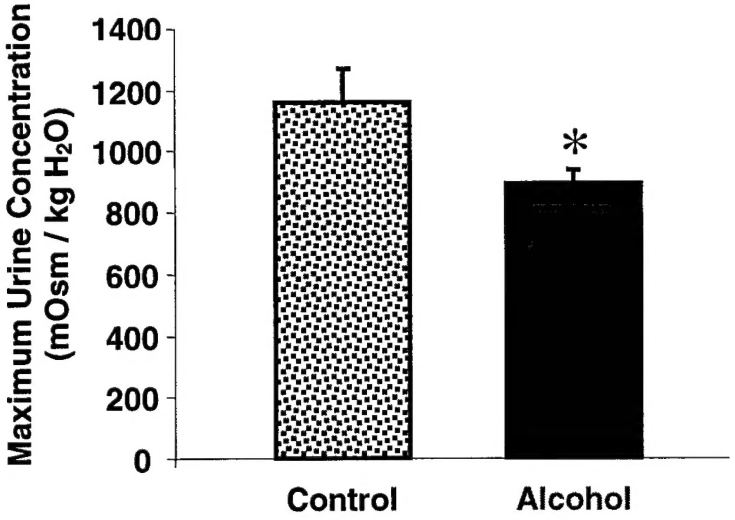


fig. 3 Maximal urine concentrating ability in rats during alcohol withdrawal.

Rats undergoing withdrawal from alcohol (n=6) exhibit reduced maximal urine concentrating ability compared to control rats (n=5).

Examination of the stimulation of vasopressin release in response to a salt load:

Preliminary results examining the relationship between baseline circulating vasopressin levels and plasma osmolality in the chronic alcohol group so far suggest that this relationship is altered in chronic alcohol exposure. We will continue to better define this relationship between vasopressin regulation by osmotic stimulation with the salt loading studies over the next year.

Assessment of vasopressin clearance to assess the influence of alcohol on vasopressin metabolism:

Because we have found that there is no difference in circulating vasopressin levels between control and alcohol exposure groups, we have focused our attention on the mechanisms of vasopressin action on the kidney as seen in the renal receptor mRNA and binding data below. However, we continue to explore the relationship between vasopressin synthesis and release by the brain into the circulation and will continue examining the factors regulating this steady vasopressin circulating level by examining vasopressin clearance in the last year of the study. It is possible that vasopressin metabolism by the kidney may be altered, causing circulating levels to be maintained independent of any change in brain vasopressin mRNA expression and vasopressin synthesis. If renal vasopressin receptors are somehow affected by chronic alcohol exposure, it is likely that clearance of vasopressin from the circulation will also be affected. This is in accordance with the theory that vasopressin renal clearance is receptor mediated (Keeler et al., 1991).

2. *In vitro assessments of tissues and samples to elucidate mechanisms behind altered fluid handling*

Measurement of vasopressin levels in the pituitary, blood, and urine:

Chronic alchoholism associated with water retention is supposedly due to increased circulating vasopressin or no change in vasopressin levels but an increase in renal vasopressin sensitivity, impaired renal water excretion, hyponatremia, and cirrhosis of the liver. Alcohol withdrawal, especially in patients with delirium tremens (Trabert et al., 1992) is linked to an increased plasma vasopressin concentration believed to be the result of rebound secretion of vasopressin. The mechanism behind increased circulating vasopressin levels is not clear because the relationship between vasopressin gene expression in the brain, synthesis, and release has not been systematically studied during the various phases of alcohol exposure and withdrawal.

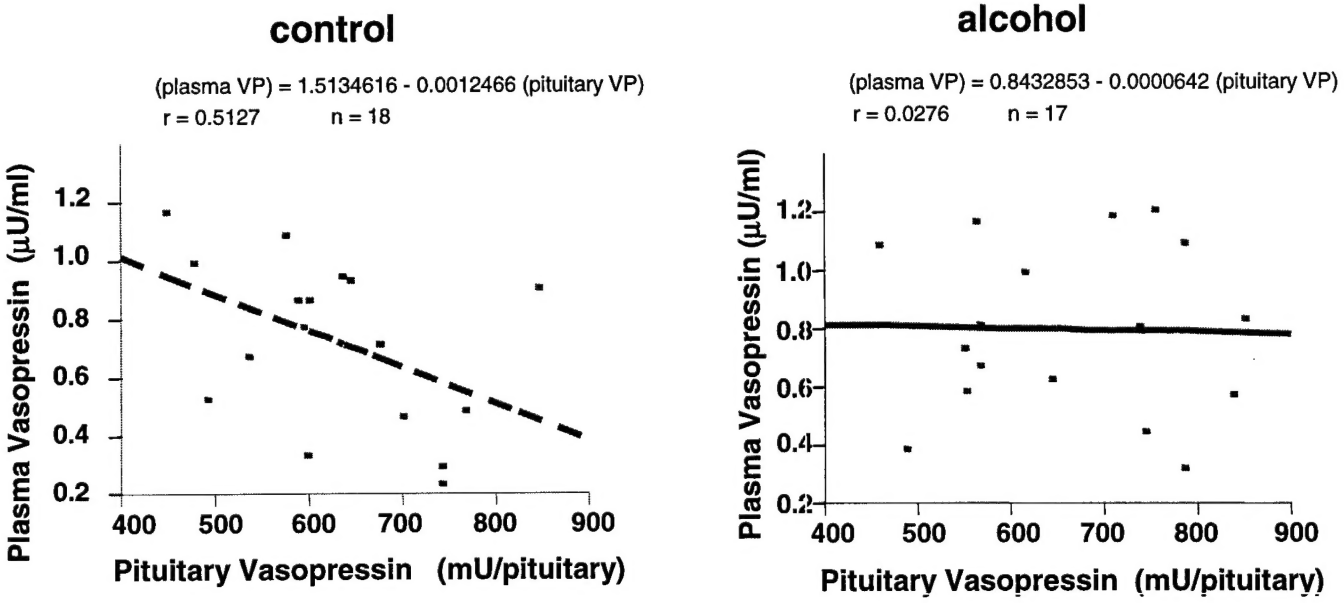


fig. 4 Relationship between pituitary and plasma vasopressin levels .
The inverse relationship between pituitary vasopressin stores and circulating vasopressin seen in control rats (n=18) is disrupted in rats exposed to alcohol (n=17).

We have begun to analyze and compare basal vasopressin levels in acute alcohol, chronic alcohol, and control groups. Preliminary assessment of pituitary vasopressin content and circulating vasopressin levels indicate that the relationship between vasopressin release and circulation is altered with alcohol exposure. In control rats, there is an inverse relationship between plasma vasopressin and pituitary content of vasopressin (fig. 4). This is similar to the relationship previously described in examining the effects of dehydration on vasopressin synthesis and release (Majzoub J.A., 1985). Further, an uncoupling of vasopressin secretion and release into the circulation has been indicated in at least one study where plasma vasopressin levels and plasma osmolality were increased while hypothalamic vasopressin mRNA remained unchanged (Hoffman and Dave, 1991). Another study has also described a disturbance in the rat hypothalamic-pituitary-adrenal axis with just acute alcohol exposure (Lee and Rivier, 1997). Additional assessment of the relationship between vasopressin brain content and blood levels

throughout the next year from all rats studied will help us better define this apparent uncoupling of vasopressin release and blood levels that occurs with alcohol exposure.

Measurement of kidney vasopressin receptor mRNA:

Our data from the water load and V2 antagonist experiments are fitting nicely in support of the idea of up or down regulation of vasopressin receptors in the kidney.

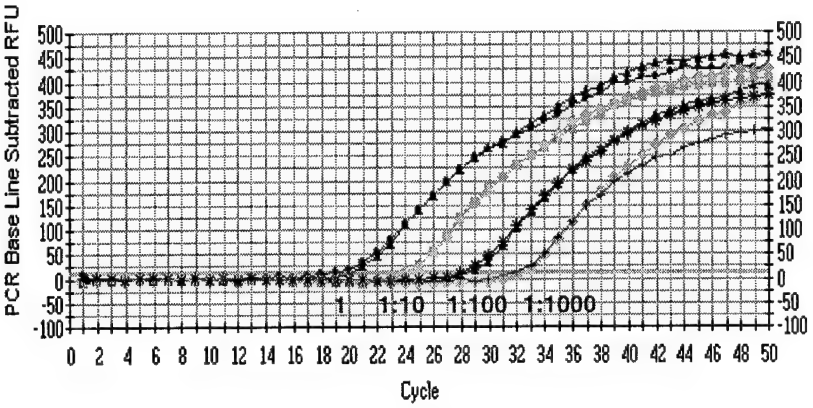
We have developed new assays for the real-time quantitative PCR (qPCR) of mRNA levels for both vasopressin and vasopressin receptors with the design of specific primers and probes. Figure 5 shows representative generation of a standard curve for the assessment of receptor mRNA copy numbers and the single band PCR product characterized by gel electrophoresis. Copy numbers derived from the threshold cycle for PCR product generation for vasopressin, vasopressin receptor, or β-actin housekeeping genes are determined and the mRNA quantity in each sample is expressed as a ratio of the gene of interest (vasopressin, V1 receptor (V1R), or V2 receptor(V2R)) per housekeeping gene. These highly sensitive and specific assays allow us to discern fine differences in mRNA which affords us a powerful tool for comparing synthesis of vasopressin and vasopressin receptors in acute alcohol, chronic alcohol, alcohol withdrawal, and control groups.

Because our results from the whole animal experiments clearly indicated that changes in renal vasopressin receptors in the different phases of alcohol exposure was likely, we first focused our attention on assessing vasopressin V2 receptor mRNA levels in the inner medullary collecting duct. Because of the highly sensitive and reproducible measurements obtained with the real time PCR technology, we were able to detect the effects of acute alcohol, chronic alcohol, and alcohol withdrawal on renal V2 receptor synthesis that would not have been as easily detected by any other method.

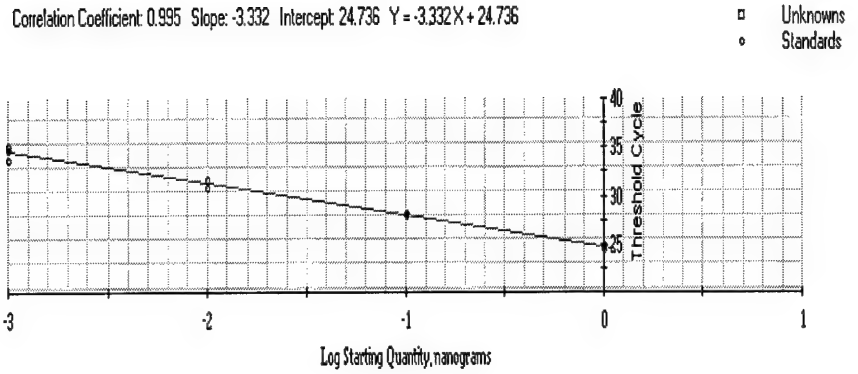
The results of renal V2 mRNA quantitation can be seen in figure 6. V2 receptor synthesis as indicated by the V2 to β-actin mRNA ratio was significantly less in rats acutely exposed to alcohol (n = 10) compared to controls (n = 10). A down regulation of renal V2 receptors is consistent with the increased water diuresis observed in the acute alcohol group. In contrast, renal V2 receptor mRNA was greater in the chronic alcohol exposure group (n = 12) compared to controls (n = 12), which is consistent with an up regulation of V2 receptors causing the impaired ability to excrete a water load with chronic alcohol exposure. Lastly, during the withdrawal phase, V2 receptor mRNA levels returned toward control levels as did water load excretion ability. Thus, these results indicate that the up regulation of renal V2 receptor mRNA seen with chronic alcohol exposure can be reversed upon withdrawal, similar to recently reported recovery of vasopressin mRNA in the brain seen with alcohol withdrawal (Silva et al, 2002).

We are excited about the ability to detect these differences and using the qPCR technology to assess vasopressin receptor numbers as the amount of tissue and numbers of animals needed for these assessments is much less than that of traditional receptor binding methods.

fig. 5 Real-time quantitative PCR generation of standard curves for the measurement of vasopressin receptor mRNA



Real time PCR rat vasopressin receptor threshold cycle vs. fluorescence



Standard curve from serial dilution of rat kidney

Electrophoresis (agarose gel) of vasopressin V1a receptor on various rat tissues

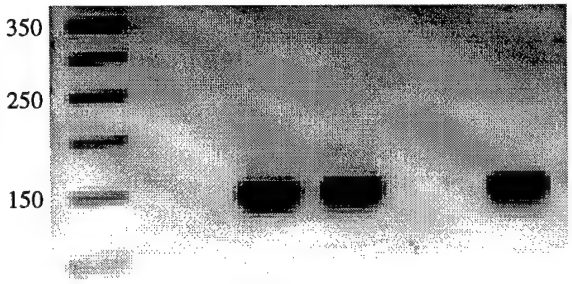
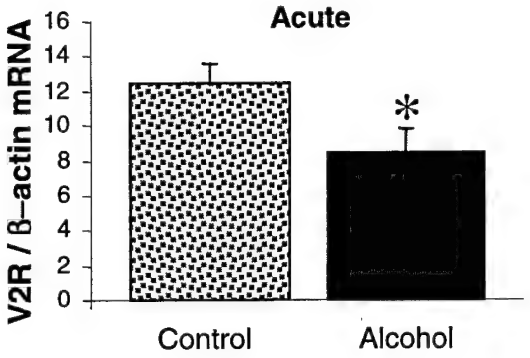
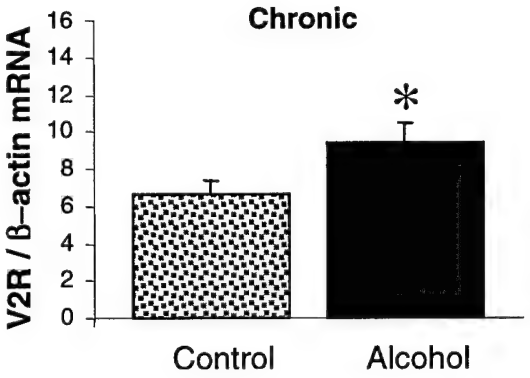
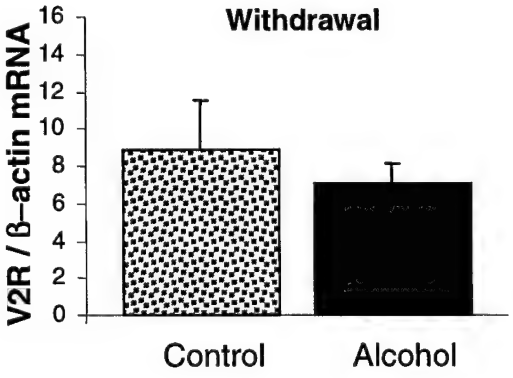


fig. 6 Vasopressin V2 mRNA in renal inner medullary collecting ducts.

Renal V2 receptor mRNA was decreased in rats with acute alcohol exposure (n = 10) compared to controls (n = 10), whereas rats with chronic alcohol exposure (n = 12) exhibited increased renal V2 mRNA compared to controls (n = 12). The upregulation of V2 receptors with chronic alcohol exposure was reversed with 4 weeks of alcohol withdrawal as there was no difference in rats undergoing alcohol withdrawal (n = 6) compared to controls (n = 6).







Assessment of kidney vasopressin receptor numbers and binding affinity:

While we initially intended on assessing vasopressin receptor numbers and binding affinity with traditional receptor binding methods, we hope to replace the need to do so with the molecular methodology of the qPCR assays we developed. However, we need to first demonstrate that the mRNA assessments can be interpreted to directly translate into receptor protein synthesis and thus receptor numbers. So far the binding studies examining vasopressin V2 receptor numbers and binding affinity are in accordance with the findings of the vasopressin V2 receptor mRNA measurements. As can be seen in figure 7, maximal V2 receptor binding in renal inner medullary collecting duct cells obtained from rats acutely exposed to alcohol is less than that of control rats, similar to the finding of reduced V2 mRNA with acute alcohol exposure.

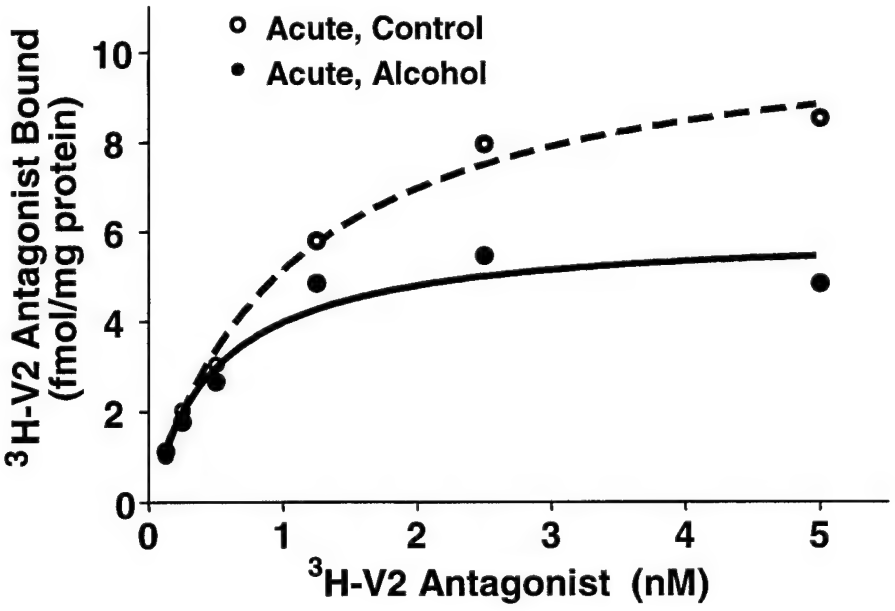


fig. 7 Competitive renal V2 receptor binding results.
Acute alcohol exposure resulted in less maximal numbers of V2 receptors in innermedullary collecting duct cells than controls.

Measurement of brain vasopressin and vasopressin receptor mRNA

Others have described decreases in vasopressin mRNA and vasopressin neurons as a result of chronic alcohol exposure (Sanna et al, 1993; Gulya et al., 1991; Harding et al, 1996; Ishizawa et al, 1990). Our preliminary results thus far confirm this reduction of vasopressin mRNA in the brain, and also reveal a suppression of V1 receptor mRNA in the brain as well. We will continue to characterize the relationship between vasopressin synthesis, brain V1 receptor regulation, and osmolality.

Further, over the next year, we also will examine the relationship between changes in vasopressin receptor mRNA in the kidneys with vasopressin and vasopressin receptor mRNA in the brain. One study has suggested that stimulation of renal mechanoreceptors appear to regulate the responses of vasopressin neurons in the paraventricular nucleus of the brain (Ciriello, J., 1998). It will be interesting to discover that up or down regulation of receptors in the kidney may influence the regulation of vasopressin receptors or vasopressin synthesis in the brain.

Assessment of kidney collecting duct cell function:

So far, our quantitative assessment of V2 receptor changes are in accordance with whole animal assessment of renal water handling abilities in all phases of alcohol exposure. Thus, it is no longer critical to verify whether changes in receptor numbers translate into changes in renal cell function. However, we will run a few experiments assessing collecting duct cell function *in vitro* to help verify and localize the altered renal sensitivity to vasopressin to the collecting duct.

Evidence of alcohol-induced altered regulation of vasopressin synthesis and release.

In the last year of this grant we will further examine the regulation of vasopressin synthesis and release. Our preliminary results thus far suggest that vasopressin synthesis (as indicated by vasopressin mRNA and pituitary content) and release (as indicated by circulating levels) are uncoupled in alcohol exposure. We have also discovered that changes in V1 receptor mRNA in the brain may be linked to plasma osmolality. We will compare our results to what is known about vasopressin mRNA expression in response to other conditions such as salt loading (Sherman, 1985) and the brain morphological changes described in other models of chronic alcohol exposure (Maderia, et al, 1993; Ruela et al, 1994).

Also, while we have found no detectable difference in vasopressin circulating levels between control and alcohol exposed rats, it may be that vasopressin levels are maintained despite altered rates of synthesis and release because clearance of the hormone from the blood is likewise altered. The whole animal studies of vasopressin clearance in the last year will help elucidate this last piece of the puzzle characterizing the effects of alcohol exposure on vasopressin regulation and action.

KEY RESEARCH ACCOMPLISHMENTS:

- Results indicate that even short-term alcohol abuse, equivalent to 3 days of binge drinking, can alter hydration status eighteen hours after the last alcohol drink, as water diuresis appears to persist even after blood alcohol concentrations are back to undetectable levels. This suggests that soldiers need to be adequately rehydrated after any use of alcohol to avoid fluid and electrolyte imbalances that could affect soldier performance in the field.

- Persistent water diuresis appears to be caused by a down regulation of renal V2 receptors as confirmed by V2 mRNA quantitation and receptor binding studies.

- Results suggest that long-term alcohol exposure (equivalent to about 2-3 six packs of beer a day for 8 weeks) impairs the ability of the kidneys to process water. This has implications for the effect of alcohol on the regulation of body fluid balance and possibly the ability to handle renal clearance of foreign chemicals and drugs.

- Results show that chronic alcohol exposure decreases the effect of drug-induced inhibition of the hormone vasopressin on renal water handling, due to long term exposure to alcohol causing a compensatory up-regulation of renal receptors for vasopressin (as confirmed by detection of increased V2 mRNA levels), and thus an increased renal sensitivity to vasopressin. This leads to an impaired ability to excrete water and a resultant fluid and electrolyte imbalance.

- Results indicate that the up regulation of V2 receptors during chronic alcohol exposure is reversible 4 weeks after termination of alcohol exposure, indicating that impaired renal fluid handling can be reversed. This has strong implications for a recommended strategy of delaying routine field drug administration (e.g. chloroquine) for soldiers until impaired fluid handling can be reversed in order to avoid drug-induced renal toxicity that is enhanced with alcohol.

- Studies looking at the regulation of brain vasopressin synthesis indicate that alcohol use causes the relationship between brain vasopressin content and circulating vasopressin levels in the blood to be disturbed. This indicates the loss of appropriate linking of the blood levels of this important water regulating hormone with the message the brain receives to synthesize the hormone in response to altered hydration status.

- Results show that V1 receptors in the brain are regulated in response to changes in osmolality. This exciting finding may indicate a putative osmoreceptor role for V1 receptors in the brain or perhaps a role as the link between vasopressin synthesis and osmotic status.

REPORTABLE OUTCOMES

- Publications/Presentations
  - Published abstract and presentation at Experimental Biology 2001:
    CFT Uyehara, CA Burghardt, GM Hashiro, and DA Person. After effects of acute alcohol exposure on renal water handling and responsiveness to vasopressin.
    FASEB J. 15(4):A134 (Abstract 154.1), 2001 and
    J. Investigative Medicine 49(2):249A (Abstract 328), 2001.

  - Published abstract and presentation at Experimental Biology 2002:
    CFT Uyehara, CA Burghardt, DPY Cheng, GM, Hashiro, AK Sato, and JR Claybaugh. Chronic alcohol exposure causes impaired water excretion and decreased renal efficacy of a V2 antagonist. *FASEB J.* 16(5):A837-A838, 2002.

- Animal models for acute and chronic alcohol exposure and withdrawal from alcohol for precise administration of alcohol that provide a consistent response have been developed for assessment of renal fluid and electrolyte handling. These models also make efficient use of animals enabling reduction of numbers of animals used in research.

- Molecular assays utilizing quantitative PCR technology have been developed for vasopressin and vasopressin V1 and V2 receptors have been designed and established by our laboratory. These assays allow highly sensitive detection of subtle physiological changes in vasopressin receptor mRNA and protein synthesis. These assays allow uncovering physiological signals for regulation of hormone synthesis and action at the effector organ that could not previously be done with traditional methods.

- Postdoctoral fellowship pharmacology training of one new biomedical research scientist is being supported by this award

- Animal model and molecular biology methods established by this project have led to development of a fetal alcohol project and award of a septic shock grant used to support Army Graduate Medical Education residents and fellows.

CONCLUSIONS:

Despite evidence of impaired renal fluid handling, hyponatremia, and water retention in chronic alcohol exposure and during withdrawal, the renal mechanisms involved and the role of altered vasopressin action in the kidney have not been elucidated. We have previously shown that after effects of short term alcohol exposure cause rats to have an increased water diuresis in response to a water load whereas after long term alcohol exposure, rats exhibit an impaired ability to excrete a water load. Our latest data indicate that in a 4-week withdrawal phase from 8 weeks of chronic alcohol exposure, water excretion impairment persists but begins to return towards control levels.

The up- and down-regulation of renal vasopressin V2 receptors appear to be behind differentially altered renal function in different phases of alcohol exposure. Prolonged diuresis during the acute phase of alcohol exposure is associated with a decrease in V2 receptor mRNA and a down-regulation of V2 receptors. During chronic alcohol exposure, renal V2 receptor mRNA is up-regulated, indicating that increased synthesis of V2 receptors causes increased renal efficacy of vasopressin despite similar circulating vasopressin levels between alcohol-exposed and control rats. During the withdrawal phase, V2 receptor mRNA returns toward normal levels, demonstrating that chronic alcohol-induced impairment of renal water excretion is reversible.

Thus, our results to date have provided evidence of water imbalance with alcohol exposure that is due to altered function and numbers of vasopressin receptors, specifically renal V2 receptors, involved with tubular water reabsorption. Additionally, preliminary examination of the relationship between vasopressin pituitary and blood content indicates there is an uncoupling of the regulation of vasopressin release and circulating levels with alcohol exposure.

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